Yvette W. Kunz and **C. Regan.** — Histochemical investigations into the lipid nature of the oil-droplet in the retinal twin-cones of *Lebistes reticulatus* (Peters). (With 5 figures)

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Histological investigations with the light and electron-microscope refer to an oil-droplet in the twin-cone of the viviparous *Lebistes reticulatus* (Müller 1952, 1954; Berger 1965). The oil-droplet is found only in one member of each twin-cone, and is situated at the scleral end of the inner segment (fig. 2). The distribution of the oil-droplet containing cones is regular within the cone-mosaic (see fig. 3 in Kunz and Wise 1973). According to Berger (1965) the droplets are colourless.

Oil-droplets have not been observed in any other teleostean retina. They are, however, characteristic of many amphibian, reptilian and avian cones, where they are yellow or orange or red, or colourless. Coloured droplets are associated with diurnal vision; colourless droplets are found in nocturnal, crepuscular and fossorial species. Light must pass through the oil-droplets to enter the sensitive cone outer segments. The coloured droplets are, therefore thought to constitute intraocular filters and as such to increase contrast, reduce glare and lessen chromatic aberration, which would result in an increased visual acuity (Walls 1942). The colourless droplets may serve as focusing lenses (Detwiler 1943). In the chick retina three types of oil-droplets — red, orange and greenish-yellow — are found. Retinal extracts yielded three different carotenoids; it was concluded that each cone concentrates one type of carotenoid in its droplet. A similar situation was found in the retina of the turtle *Clemmys insculpata* (Wald and Zussman 1937, 1938). When the oil-droplets begin to appear in the chick embryo (13/14th day) they are colourless; they become coloured on the 15th day (Coulombre 1961).

Since the oil-droplet in *Lebistes reticulatus* has been so named without proof of its lipid nature, we decided to subject it to various histochemical tests.

Material and Methods

Fixed, as well as unfixed, kryostat sections of whole heads were used. Different staining methods were employed according to the diagram shown below (fig. 1). Unless otherwise stated, the eyes used were light-adapted. In this condition, the

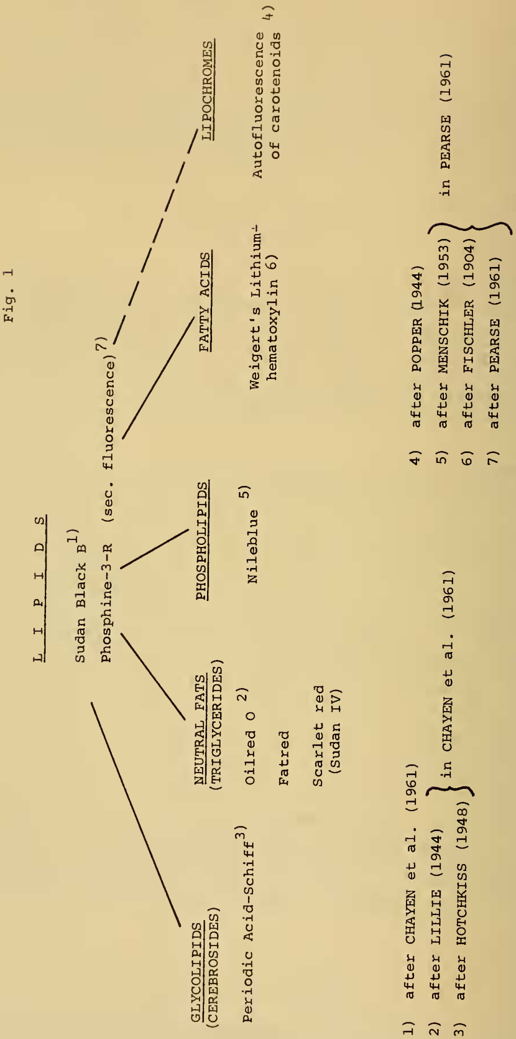


Fig. 1
LIPIDS
Sudan Black B 1)
Phosphine-3-R (sec. fluorescence) 7)
GLYCOLIPIDS (CEREBROSIDES)
NEUTRAL FATS (TRIGLYCERIDES)
PHOSPHOLIPIDS
FATTY ACIDS
LIPOCHROMES
Periodic Acid-Schiff 3)
Oilred O 2)
Fatred
Scarlet red (Sudan IV)
Nileblue 5)
Weigert's Lithium-hematoxylin 6)
Autofluorescence of carotenoids 4)

1) after CHAYEN et al. (1961)
2) after LILLIE (1944)
3) after HOTCHKISS (1948)
} in CHAYEN et al. (1961)

4) after POPPER (1944)
5) after MENSCHIK (1953)
6) after FISCHLER (1904)
7) after PEARSE (1961)
} in PEARSE (1961)

FIG. 1.

Histochemical methods used for lipid tests.

ellipsoids of the rods are overlaid with pigment granules, and their staining reaction cannot, therefore, be ascertained.

RESULTS

The first stain employed was Sudan Black B, a general lipid stain. Moderate staining was observed in the rod outer segments. In the cone outer segments and ellipsoids the staining was weaker. This stain did not show the oil-droplet (fig. 3). Treatment with Phosphine-3-R, a fluorochrome for lipids, showed a strong reaction in the rod outer segments and in the cones. The region of the oil-droplet did not react differently. Autofluorescence, which would be expected from carotenoids dissolved in lipids, did not occur in the region of the oil-droplets.

Although the results obtained so far were negative with regard to the oil-droplet, we proceeded to stain for specific lipid components. Oil red O was shown to react only with the outer segments of the rods; the cones did not retain the stain. The results with Fatred and Scarlet Red/Sudan IV corresponded with those obtained with Oilred O. With Nileblue, the outer segments of the rods and cones stained strongly and the ellipsoids of the cones weakly. The oil-droplet was not evident (fig. 4). Periodic Acid-Schiff failed to stain the cones. A reaction was observed, however, in the outer segments of the rods. Weigert's Lithium Hematoxylin did not react with the photoreceptors.

Unmasking of the lipids was now attempted. The "burnt Sudan Black B" method yielded a strong reaction in the rod outer segments and a moderate reaction in the cones. The coloration was much more pronounced than with the ordinary Sudan Black B method. For the first time the oil-droplet became visible, as a colourless vacuole bounded by a distinct membrane. The dark-adapted eye was also tested with the "burnt Sudan Black B" method. The rod outer segments stained heavily and the ellipsoids lightly. The cones showed also a positive reaction. The region of the oil-droplet could not be tested since it was occluded by the rods (which move vitreally during dark-adaptation).

To summarise our results: it emerges that the cones of *Lebistes* stain diffusely for lipids (probably phospholipids), without selectively staining the region of the structure hitherto called oil-droplet. This brings *Lebistes* back into line with all other teleosts tested. According to WALLS (1942) the oil-droplet was "lost" during the evolution of fishes: oil-droplets are present in the photoreceptors of the coelacanths, dipnoi and chondrostei, but they are absent in the holostei and teleostei.

What then is this large, spherical structure at the scleral end of the ellipsoid? From our preliminary electron-microscopical investigations it would seem that it could be a modified, giant mitochondrion (KUNZ and WISE, 1973). Retinal smears, subjected to Janusgreen B, a vital stain for mitochondria, showed that the "oil-droplet" stains similarly to the ellipsoidal mitochondria (fig. 5). Future lines to be pursued will, therefore, involve the isolation of total mitochondrial fractions and subsequent segregation of these fractions in an attempt to obtain an "oil-droplet"-rich fraction. This would enable a comparison — between "oil-droplets" and normal mitochondria — of characteristics such as oxygen utilisation, spectra difference and DNA- and RNA-composition.

ZUSAMMENFASSUNG

Die sogenannte Oelkugel im Zwillingszapfen der *Lebistes*-Retina wurde histochemisch untersucht. Keine der gebräuchlichen Methoden ergab eine positive Reaktion für Fettstoffe. Es wird deshalb vorgeschlagen, dass es sich bei der "Oelkugel" um ein, vielleicht spezialisiertes, Riesen-Mitochondrion handeln könnte.

FIG. 2.

Micrograph of *Lebistes* retina, stained with toluidene blue, to show oil-droplets in twin-cones (outer cones).

FIG. 3.

Kryostat section of same region as in fig. 2, stained with Sudan Black B.

FIG. 4.

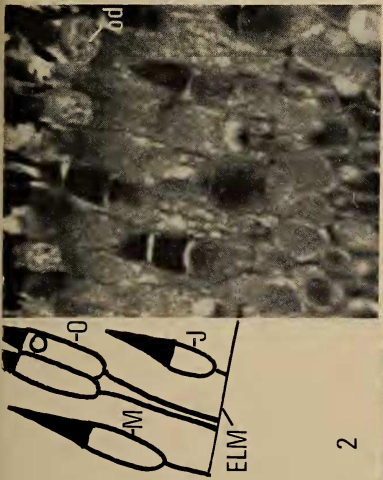
As fig. 3 but stained with Nileblue.

FIG. 5.

Retinal smear treated with Janusgreen B.
Note staining of mitochondria and oil-droplet.

ELM = external limiting membrane
J = inner cone
M = middle cone
mi = mitochondrion

O = outer cone
od = oil-droplet
pe = pigment epithelium



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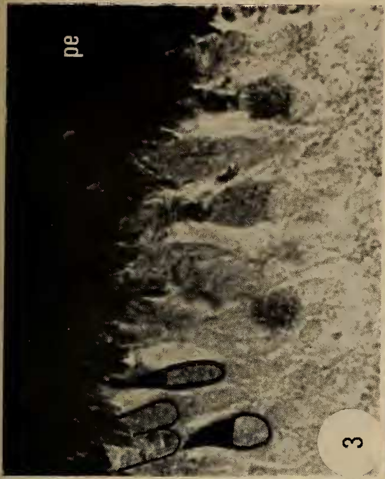

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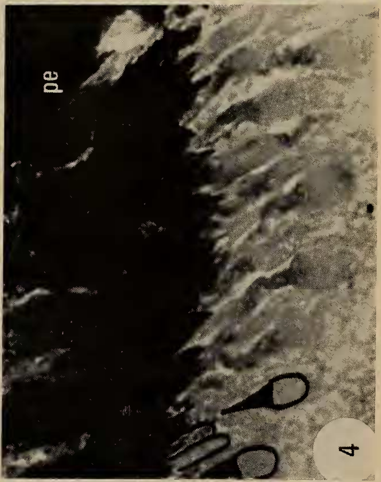

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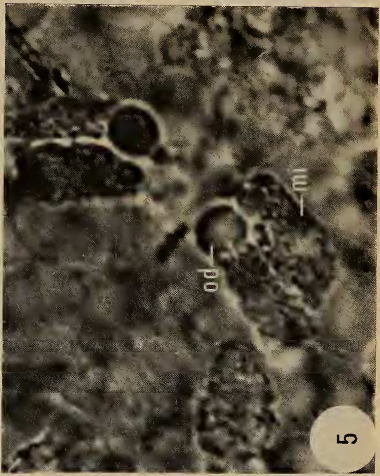

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